AMENDMENTS TO THE SPECIFICATION

The amendments to the specified portions of the specification will replace all previous versions of that portion of the specification. No new matter has been introduced by way of the amendments to the specification.

Page and paragraph references cited hereinbelow are taken from United States Patent publication 20060217317.

Amended Paragraph [0010] on Page 1

Please replace paragraph [0010] on page 1 with the following replacement paragraph. Amended paragraph [0010] adds SEQ ID NOs. 1 – 14 referenced in Figure 1. Amended paragraph [0010] also contains a correction of a minor grammatical error therein.

[0010] The present invention provides a novel class of oligopeptides that include amino acid sequences containing cleavage sites for human glandular kallikrein (HK2) (FIG. 1) (SEQ ID NOs:1-14). These cleavage sites are derived from an hK2 specific cleavage map of semenogelin I and II (FIG. 1) and from hK2 cleavable peptides isolated from a random peptide library. These oligo[[-]]peptides are useful in assays that can determine the free hK2 protease activity. Furthermore, the invention also provides a therapeutic prodrug composition, comprising a therapeutic drug linked to a peptide, which is specifically cleaved by hK2. The linkage substantially inhibits the non-specific toxicity of the drug, and cleavage of the peptide releases the drug, activating it or restoring its non-specific toxicity.

Amended Paragraph [0018] on Page 2

Please replace paragraph [0018] on page 2 with the following replacement paragraph. Amended paragraph [0018] contains SEQ ID NO. references for the peptide sequences listed in FIG. 1 and a new SEQ ID NO. reference for the sequence NO_2-Y-G-K-A-X_1-X_2-X_2-X_3-Dap-F-K(ABZ).

[0018] FIG. 1 depicts the amino acid sequences of peptides hydrolyzed by hK2 (SEQ ID NOs:1 – 14). A random peptide library was constructed with the sequence NO_2-Y-G-K-A-X_1-X_2-X_3-

Dap-F-K(ABZ) (SEQ ID NO:48), wherein NO_2-Y is a nitrotyrosine quencher, X_1, X_2, and X_3 are any random L-amino acid except for cysteine (n=19), Dap is diaminopropanoate, and K(ABZ) is lysine coupled to the fluorophore aminobenzoic acid (ABZ). hK2 cleavage sites are denoted by single or double slashes (/ or //). The 14 peptide sequences shown are set forth as SEQ ID NOs:1-14, respectively. SEQ ID NOs:22-35 correspond to the peptide sequences obtained when a leucine residue is added after the X_3 position of SEQ ID NOs:1-14, respectively.

Amended Paragraph [0023] on Page 2

Please replace paragraph [0023] on page 2 with the following replacement paragraph. Amended paragraph [0023] contains a new SEQ ID NO. reference for the solid-phase peptide sequence Y'GKA$X_1X_2X_2$-Dap-F-K'-PEGA.

[0023] FIG. 6 depicts the chemical structure of fluorescence-quenched combinatorial `one bead-one peptide` library [~~(~~Y'GKAXXX-Dap-F-K'-PEGA, (SEQ ID NO:49) where Y' is nitrotyrosine and K' is 2-aminobenzoic acid substituted lysine~~)~~].

Amended Paragraph [0025] on Page 2

Please replace paragraph [0025] on page 2 with the following replacement paragraph. Amended paragraph [0025] contains SEQ ID references for the peptides referenced in Figure 8.

[0025] FIG. 8 depicts HK2 mediated hydrolysis of various peptide and prodrug substrates (125 mM each) (SEQ ID NOs:18, 41, 42 and 43). Fluorescent substrates were analyzed by means of a fluorescence plate-reader (ex=355 nm, em=460 nm). acGKAFRRL-12ADT (SEQ ID NO:41) and acGKAFRRLG (SEQ ID NO:43) were analyzed by HPLC and quantified by HLPC integration.

Amended Paragraph [0042] on Page 4

Please replace paragraph [0042] on page 4 with the following replacement paragraph. Amended paragraph [0042] corrects the capitalization state of the term cisplatinum.

[0042] Certain therapeutic drugs contain primary amines and are among the preferred agents. These include the anthracycline family of drugs, vinca drugs (e.g., vinca alkaloids such as

vincristine, vinblastine, and etoposide), mitomycins, bleomycins, cytotoxic nucleoside analogs (e.g., 5-flurouracil, gemcitabine, and 5-azacytidine), the pteridine family of drugs, diynenes, podophyllotoxins, antiandrogens (e.g., biscalutamide, flutamide, nilutamide, and cyproterone acetate), antifolates (e.g., methotrexate), topoisomerase inhibitors (e.g., Topotecan and irinotecan), alkylating agents (e.g., cyclophosphamide, ~~C~~cisplatinum, carboplatinum, and ifosfamide), taxanes (e.g., paclitaxel and docetaxel), and compounds which are useful as targeted radiation sensitizers (e.g., 5-flurouracil, gemcitabine, topoisomerase inhibitors, and cisplatinum). Additional particulary useful members of these classes include, for example, doxorubicin, daunorubicin, carminomycin, idarubicin, epirubicin, aminopterin, methopterin, mitomycin C, porfiromycin, cytosine arabinoside, melphalan, vindesine, 6-mercaptopurine, and the like, including any therapeutic drug (e.g., any therapeutic drug used in the treatment of cancer, including prostate and/or breast cancer) known to those of skill in art.

Amended Paragraph [0074] on Page 7

Please replace paragraph [0074] on page 7 with the following replacement paragraph. Amended paragraph [0074] contains a new SEQ ID NO. reference for the solid-phase peptide sequence FmocX_1-X_2-X_3-Dap-Phe-K(Abz)-PEGA.

[0074] Amino acid couplings were performed according to established Fmoc/tBu protocols using Hobt/DIC activation (Chan, W. and White, P. Fmoc solid phase peptide synthesis, a practical approach. New York: Oxford University, 2000) and performing standard double couplings. Generally, completion of acylation reactions was verified by both Ninhydrin (Kaiser, E. et al. (1970) Anal. Biochem. 34:595-598) and fluorescamine testing (Felix, A. M. and Jimenez, M. H. (1973) Anal. Biochem. 52:377-381). Deprotection of the side-chain protecting group was performed by using Reagent K (TFA/thioanisole/water/phenol/EDT 82.5:5:5:5:2.5 v/v). The Fmoc protecting group was removed with 25% piperidine in DMF. N-α-Fmoc-N-βO-t.-Boc-L-diaminopropionic acid (Fmoc-Dpr(Boc)-OH, Novabiochem) was used for the introduction of Dap. Three randomized positions were introduced using a Labmate parallel Organic Synthesizer (4x6 vessels, Advanced Chemtech, Louisville, Ky.) according to the split-and-mix procedure (Houghten, R. A. (1985) Proc. Natl. Acad. Sci. USA 82:5131-5135). All natural amino acids, except for cysteine were used with the following side-chain protection: Trt (Asn, Gln, His), tBu

(Tyr), OtBu (Asp, Glu, Ser, and Thr), Boc (Lys, Trp) and Pmc (Arg). Amino acid stock solutions (0.5 M with 0.5 M Hobt) were mixed with DIC for 20 min (4 Eq. of each). The activated amino acids were added to the resin and 0.15 ml of 5% DIEA in DMF was added. After 2-3 hrs, the resin aliquots were washed (3xNMP, 3xMeOH, 3xDMF) and couplings were repeated with 2 eq. amino acid for 1-2 hrs. A resin sample of each aliquot was subjected to a Ninhydrin and a fluorescamine test which showed completion of the acylation reactions in all cases. Next, the resin aliquots were pooled [{FmocX$_1$-X$_2$-X$_3$-Dap-Phe-K(Abz)-PEGA}] (SEQ ID NO:50) was deprotected with piperidine and the remaining four constant residues, alanine, lysine, glycine and nitrotyrosine (Y', Fluka) were added as Fmoc amino acids in batch with Hobt/DIC activation as described above. For the final deprotection of the side chains, the resin was suspended in Reagent K (1x10 min, 1x3 hrs). The resin was washed with 95% acetic acid (3x), DCM (3x), DMF (3x), 5% DIEA in DMF (3x), and DMF (6x). The resin was stored until screening suspended in DMF at -20°C.

Amended Paragraph [0080] on Page 8

Please replace paragraph [0080] on page 8 with the following replacement paragraph. Amended paragraph [0080] contains a new SEQ ID NO. reference for the sequence ac-GK(ivDde)AFRRL.

[0080] Deprotection of the acid-labile protecting groups and purification was performed as outlined above. Boc-12 ADT was synthesized as previously described (Jakobsen, C. M. et al. (2001) J. Med. Chem. 44:4696-4703). TFA treatment, followed by semi-prep HPLC and lyophilisation afforded the amine containing 12ADT. The protected peptide [{ac-GK(ivDde)AFRRL}] (SEQ ID NO:51) was coupled to 12ADT after Hobt/DIC activation. After completion of the reaction, the ivDde group was removed by adding hydrazine to the reaction mixture (2% final, 30 min). Semi-preparative HPLC yielded acGKAFRRL-12ADT (SEQ ID NO:41), typically in 60-70% yield. Product was confirmed by MALDI-TOF analysis.

Amended Section Heading Preceeding Paragraph [0081]

Please replace the section heading preceeding paragraph [0081] as follows to identify the SEQ ID NO. reference for the sequence ac-GKAFRR-L12ADT.

Determination of Plasma Levels of ac-GKAFRR-L12ADT (SEQ ID NO:41)

Amended Paragraph [0086] on Page 9

Please replace paragraph [0086] on page 9 with the following replacement paragraph. Amended paragraph [0086] contains a new SEQ ID NO. reference for the solid-phase peptide sequence Y'GKAFRLK'-PEGA.

[0086] These studies suggested that alternative resin supports and/or linkers would be required to generate libraries for screening purposes. Synthesis of a test hK2 peptide substrate on polyethylene glycol A (PEGA) resin [(Y'GKAFRLK'-PEGA, (SEQ ID NO:52) where Y' is 3-nitrotyrosine and K' is Lysine(Abz))] demonstrated the importance of the resin support since this peptide was digested to yield fluorescent beads after 10-12 hours of incubation with 4 μg/ml hK2.

Amended Paragraph [0087] on Page 9

Please replace paragraph [0087] on page 9 with the following replacement paragraph. Amended paragraph [0087] contains a new SEQ ID NO. reference for the solid-phase peptide sequence Y'GKAFRL-Dap-F-K'-PEGA.

[0087] Additionally, Walle and co-workers (Thorpe, D. S. and Walle, S. (2000) Biochem. Biophys. Res. Commun. 269:591-595) published data from a small combinatorial library to find linkers for the optimal display of peptide ligands to various protein targets. They reported that the insertion of a dipeptide consisting of a cationic residue together with a hydrophobic residue presents a general method for optimizing peptide display on solid phase beads. To test this observation in the system used herein, a linker described by Walle et al., in which the cationic residue was diamino propanoic acid (Dap) and the hydrophobic residue was phenylalanine (F), was incorporated. The test peptide Y'GKAFRL-Dap-F-K'-PEGA (SEQ ID NO:53) was synthesized and observed that the time required to generate clearly detectable fluorescence on the beads was reduced to 4-5 hours compared to 10-12 hours for the same peptide sequence lacking the Dap-F linker. On the basis of these results, the Dap-F dipeptide linker was included in all subsequent libraries. The final structure of the library is shown in FIG. 6.

Amended Paragraph [0088] on Page 9

Please replace paragraph [0088] on page 9 with the following replacement paragraph. Amended paragraph [0088] adds the SEQ ID NO. reference for the sequence Y'GKAX$_1$X$_2$X$_3$-Dap-F-K'-PEGA. Amended paragraph [0088] also contains a correction of a minor grammatical error.

[0088] The final library used for screening with hK2 contained the general sequence Y'GKAX$_1$X$_2$X$_3$-Dap-F-K' PEGA (SEQ ID NO:49) where X=any of 19 amino acids (cysteine was excluded from library) and contained 193 sequences on ~50,000 beads (i.e. ~7 beads for each unique peptide sequence). After carefully removing any false positive fluorescent beads from the library (~40-50 beads), purified, enzymatically active hK2 was added at a final concentration of 4 μg/ml. After 1 hour, the first positive bead was removed. Over the subsequent 3 hours, 9 more beads were selected. In total, 14 beads were selected over a period of 24 hours. Positive beads were sequenced by Edman degradation. The sSequences of the fourteen peptides are shown in FIG. 1.

Amended Table I on Page 11

Please replace Table 1 on page 11 with the following replacement table and following text. Amended Table 1 adds SEQ ID NO. references for the peptide sequences Abz-GKAFRRLY' and GKAFRRL12ADT.

Table I

Relative hydrolysis rates normalized to the enzyme with the lowest hydrolysis rate

Protease	Relative hydrolysis rate	
	Abz-GKAFRRLY' (SEQ ID NO:54)	GKAFRRL12ADT (SEQ ID NO:41)
Cathepsin D	1	1
Cathepsin B	1	1
Plasmin	36	750
hK2	353	125
Urokinase	17	1